Giant Glial Cell: New Insight Through Mechanism-Based Modeling

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Abstract The paper describes a detailed mechanism-based model of a tripartite synapse consisting of P- and R-neurons together with a giant glial cell in the ganglia of the medical leech (*Hirudo medicinalis*), which is a useful object for experimental studies in situ. We describe the two main pathways of the glial cell activation: (1) via IP_3 production and Ca^{2+} release from the endoplasmic reticulum and (2) via increase of the extracellular

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potassium concentration, glia depolarization, and opening of voltage-dependent Ca^{2+} channels. We suggest that the second pathway is the more significant for establishing the positive feedback in glutamate release that is critical for the self-sustained activity of the postsynaptic neuron. This mechanism differs from the mechanisms of the astrocyte-neuron signaling previously reported.

Keywords Tripartite synapse • Glia • Neuron • Modeling

1 Introduction

Mutual communication between neurons and glial cells has been recognized as being essential for the normal functions of the nervous system during development, signal processing, adaptation, and learning. By release of neurotransmitters and other signaling molecules, glia affect both neural excitability and synaptic transmission and thus coordinate electric activity across neuronal networks [1–3]. Investigations of Ca^{2+} wave propagation along astrocyte networks [4], nitric oxide signaling between astrocytes and blood vessels [5, 6], and related studies, convincingly show the importance of glial cells in the functional architecture of the brain.

In an attempt to understand the role of glial cells in the brain, most modeling studies of the neuro–glial interactions have focused on a particular type of glial cells, the astrocytes. Experimental data on astrocytes are often contradictory, and most researchers prefer to model a particular, known mechanism rather than effects resulting from activation of multiple pathways. Nadkarni and Jung [7] introduced the concept of a “dressed neuron” as a set of equations that describe the activation of an astrocyte by a neuron via inositol 1,4,5-triphosphate (IP_3) production, which triggers calcium release from the endoplasmic reticulum (*ER*). The increase in the intracellular Ca^{2+} concentration, in its turn, causes glutamate outflux from the astrocyte to the neuron. Such bidirectional communication can provide long-term potentiation and persistent spiking of the “dressed” neuron. Postnov et al. [8] have developed a more general, though still simplified, model of neural–glial ensembles that is useful in the study of the responses of different types of glial cells without focusing on particular animal species.

For a comprehensive understanding of glia function and its effects on neuronal activity, it is crucial to develop mathematical models that account for multiple pathways of inter- and intracellular signaling. In this paper, we introduce a mechanism-based model of a tripartite synapse. This structure, which contains pre- and postsynaptic neurons and a glial cell, is found in simple nervous systems, frequently used in studies of the physiological properties and function of glial cells in general [9].

The nervous system of the medical leech (*Hirudo medicinalis*) serves as an experimental model system to explore basic principles and phenomena in synaptic transmission and the function of neuronal networks. The advantages of this system include the simplicity of its structure, with a small number of neurons and glial cells, and its well-known neuronal circuitry and the analogy of its cellular pathways and signaling mechanisms with their counterparts in mammals [10]. Therefore, leech glial cells and identified neurons with known synaptic properties represent an attractive preparation for neurophysiological research, mathematical modeling, and studies of the role of glia in synaptic transmission. We have previously analyzed the role of variations in the extracellular potassium concentration in synchronizing small ensembles of neurons [11, 12]. In the present paper, we will model

and test different pathways of activation of the postsynaptic R-neuron and giant glial cell (GGC) and its response to different stimuli. We propose that there is an additional mechanism for long-term potentiation of a postsynaptic neuron induced by a glial cell. To simplify the full-scale description of the model, we present it module by module and summarize the parameter and constant values in the Section 5.

2 Model Description

The system under consideration represents an identified synapse in the medical leech ganglion, consisting of a presynaptic P (pressure)-neuron, a postsynaptic R (Retzius)-neuron, and a GGC. The latter is similar in many ways to astrocytes and is noteworthy by its huge dimensions (cell body diameter is about $100\text{ }\mu\text{m}$) and special location. In the leech ganglia, the two GGC are the only cells that are located in the center of the ganglion and are surrounded by a meshwork of dendrites, axons, and synaptic contacts of neurons [13, 14]. Thus, GGCs provide a sheath for all synapses that, to some extent, can be compared with the network of astrocytes.

2.1 Extracellular Potassium and Glutamate Signaling

In the model description below, we refer to the quantitative mathematical model of synaptic signal transmission between the P- and R-neurons of the medical leech (Postnov et al., manuscript in preparation), which is an essential part of the tripartite synapse model under study. Because of space limitations, we cannot provide a full description here.

The P-neuron responds to an external pressure of about 20 g/cm^2 applied to the skin receptors with a spike train consisting of 3–10 spikes with an action potential duration of 30–40 ms [10]. Depolarization of the P-neuron presynaptic terminal results in glutamate (Glu) release. Glu diffuses to the R-neuron membrane and interacts with the ionotropic AMPA-receptors on it. This is followed by the depolarization due to the influx of Na^+ ions [15]. Figure 1 shows the tripartite synapse with its P- and R-neurons surrounded by the GGC. The figure also illustrates some of the main pathways and processes involved in the activation of and communication among the three cells.

Depolarization of the R-neuron (postsynaptic) membrane leads to action potential generation and the activation of voltage-dependent K-channels. This again results in a pronounced K^+ outflux in the case of prolonged spike trains (Fig. 1). It is essential that, under high synaptic transmission and neuronal activity, the concentration of K^+ ions rises considerably in the intercellular volume W . This modifies the properties of the surrounding neurons and glial cells [16].

Glutamate, released from the P-neuron, also interacts with metabotropic glutamate receptors (m-GluRs) on the GGC [14]. A cascade of intracellular processes in GGC, evoked by high concentrations of extracellular K^+ and Glu, results in the release of glutamate from GGC into the same extracellular volume W (described in detail in the next section).

The elevated concentration of the “glial” glutamate can both inhibit (to some extent) the presynaptic activity and provide an additional excitatory stimulus to the R-neuron (Fig. 1) [15, 17].

Fig. 1 Illustration of the tripartite synapse with its P- and R-neurons and the GGC (color on-line only). *Blue circles* represent glutamate (*Glu*) released from presynaptic P-neuron under excitation. Glutamate interacts with ionotropic receptors (*i-GluR*) of the R-neuron and metabotropic receptors (*m-GluR*) of the GGC. Activation of *m-GluRs* results in IP_3 production and opening of IP_3 -receptors (IP_3R). *Grey circles* are glutamate released from the glial cell during increases in the cytosolic Ca^{2+} concentration above a certain threshold. The main entry of Ca^{2+} into the glial cytoplasm occurs via Ca^{2+} -channels. “Glial” glutamate interacts with both *i-GluRs* of the R-neuron and *m-GluRs* of the glial cell. For a detailed description, see the main text

Based on the above discussion, the equation for extracellular potassium concentration $[K]_W$ is formulated as:

$$W \frac{d[K]_W}{dt} = (I_{KR} + I_{KG})/F - J_{\text{pump}} + \gamma_W([K]_0 - [K]_W), \quad (1)$$

where I_{KR} is the outward potassium current from the R neuron and I_{KG} is the outward potassium current from the glial cell. F is the Faraday constant, J_{pump} describes the inward potassium flow to the glial cell, and $\gamma_W([K]_0 - [K]_W)$ describes the diffusion between the considered, immediate part of extracellular space W and an assumed outer bath with constant potassium concentration $[K]_0$.

For the extracellular glutamate concentration [Glu], the governing equation reads:

$$W \frac{d[\text{Glu}]}{dt} = M_s + M_G + \gamma_G([\text{Glu}]_0 - [\text{Glu}]), \quad (2)$$

where $M_s = r_s p$ describes the glutamate release during the synapse activation. r_s is the maximum release rate and p a dimensionless scaling factor. M_G is the glutamate release by the activated glial cell, and $\gamma_G([\text{Glu}]_0 - [\text{Glu}])$ describes the degradation of an excess glutamate concentration towards a small equilibrium value $[\text{Glu}]_0$. This term mainly accounts for the diffusion to the outer bath but also incorporates the contribution from different processes like uptake of excessive glutamate by the glial cell and neurons. For the sake of simplicity, we use the same value for diffusion coefficients in (1), (2), and (4), namely, the value given in the table in the Appendix, for small molecules.

The extracellular volume per cell surface area W was estimated as follows. The typical distance between the surface of GGC and the neuropil is about 15 nm [10]. This translates into $W \approx 1.5 \text{ nL/cm}^2$. Taking into account that part of this volume is filled with neuronal dendrites and axons, reasonable W values are assumed to fall in interval $[0.5; 1.5] \text{ nL/cm}^2$. In our model, the value $W = 1.0 \text{ nL/cm}^2$ is used everywhere.

2.2 Giant Glial Cell

2.2.1 Physiological Background Data

Let us recall some of the main physiological properties of the GGC of the leech (Fig. 1).

- The GGC features a large set of ion channels, receptors, and transporters. The resting membrane potential varies from -50 to -75 mV and is mainly determined by the K^+ permeability of the plasma membrane [9];
- The cytoplasmic Ca^{2+} concentration increases as the sum of two inputs: from the extracellular space via the plasma membrane and from internal stores such as the ER. (1) Ca^{2+} influx from the outside occurs mainly via voltage-dependent Ca^{2+} -channels [18], activated at membrane potentials more positive than -50 mV . Hence, membrane depolarization beyond -50 mV evokes large and rapid inward Ca^{2+} transients into the GGC. Membrane depolarization of this magnitude can be obtained by raising the external K^+ concentration above 10 mM . In ganglia of the medical leech, the extracellular potassium concentration varies from the normal level of 4 to 10 mM under conditions of high neuronal activity [14]. (2) Ca^{2+} influx from the ER occurs due to a cascade of processes in the cytoplasm. Interaction of Glu with m-GluRs of the GGC results in the production of IP_3 , which activates Ca^{2+} release via IP_3 -receptors in the ER. The activation of IP_3 -receptors is regulated by cytoplasmic Ca^{2+} , and there is a biphasic relationship between the open probability of IP_3 -dependent channels and Ca^{2+} release. Potentiating effects of Ca^{2+} on the open probability of IP_3 -channels provide a mechanism for Ca^{2+} -induced Ca^{2+} -release via IP_3 -receptors. In contrast, high levels of cytoplasmic Ca^{2+} provide a negative feedback on the release of more Ca^{2+} . The uptake of Ca^{2+} from the cytoplasm into the ER and extracellular space is mediated by Ca^{2+} -ATPases in the ER and plasma membrane. There is also a leakage Ca^{2+} current via the plasma and ER membranes.

- In contrast to Ca^{2+} signaling in mammalian astrocytes, Ca^{2+} transients in leech giant cells are mostly caused by Ca^{2+} influx from the extracellular space rather than by Ca^{2+} release from intracellular stores [18]. So far, only glutamate is known to cause intracellular Ca^{2+} release [19]. The IP_3 -sensitive Ca^{2+} release from the endoplasmatic reticulum results in a cytoplasmic Ca^{2+} rise of 10–30 nM. This is much less than IP_3 -mediated Ca^{2+} transients observed in vertebrate glial cells, where Ca^{2+} rises by one to two orders of magnitude [20]. Note that the above comments do not imply that transmembrane calcium fluxes are unimportant in vertebrate glial cells. For example, in [21], it is demonstrated that the calcium extrusion rate through the astrocyte membrane is critically involved in the generation of different firing patterns of the neuron.
- The increase in the Ca^{2+} concentration in the GGC cytoplasm triggers glutamate release into the intercellular space, presumably via exocytosis [22].

2.2.2 Mathematical Description

With a given extracellular volume per cell surface area W for the processes involved in the modeling, all equations that describe processes in the GGC are likewise formulated in terms of an intracellular volume W_G . Because of the difficulty of explicitly estimating both W and W_G , we use the simple assumption $W_G = W$ below.

IP_3 production Intracellular production of IP_3 is described as in [23] with proper account of W_G :

$$W_G \frac{d[\text{IP}_3]}{dt} = r_g \frac{[\text{Glu}]^{0.45}}{[\text{Glu}]^{0.45} + [\text{Glu}_1]^{0.45}} + \frac{1}{\tau_{\text{IP}_3}} ([\text{IP}_3]_{\text{eq}} - [\text{IP}_3]), \quad (3)$$

and with $[\text{IP}_3]$ denoting the IP_3 concentration. The first term on the right-hand side approximates experimental data on the glutamate-induced production of cytosolic IP_3 and is based on Hill's formalism with a maximum production rate r_g and half-rate glutamate concentration $[\text{Glu}_1] = 0.785 \text{ mM}$. The second term describes degradation of IP_3 with a degradation rate $1/\tau_{\text{IP}_3}$ and an equilibrium concentration $[\text{IP}_3]_{\text{eq}}$.

Calcium dynamics Description of the calcium dynamics in the GGC is based on a modification of the Li-Rinzel model following Nadkarni and Jung [7, 24]. The cytosolic calcium concentration $[\text{Ca}]$ depends on: (1) the influx of Ca^{2+} from the ER via IP_3 -dependent channels, J_{ER} ; (2) the leakage influx from the ER to the cytoplasm, J_{ER} ; and (3) the calcium flux pumped from the cytoplasm into the ER by means of Ca^{2+} -ATPase, J_{PER} . In addition, our model accounts for the following transmembrane calcium fluxes: (4) Ca^{2+} influx via the voltage-dependent Ca^{2+} -channels in the plasma membrane, J_{CM} , and (5) Ca^{2+} outflux into the extracellular space by means of Ca^{2+} -ATPase of plasma membrane, J_{PM} .

The corresponding equation for the calcium concentration reads:

$$W_G \frac{d[\text{Ca}]}{dt} = \eta_{\text{ER}}(J_{\text{ER}} - J_{\text{PER}} + J_{\text{ER}}) + (1 - \eta_{\text{ER}})(J_{\text{CM}} - J_{\text{PM}}) + \gamma_G([\text{Ca}]_0 - [\text{Ca}]). \quad (4)$$

The dimensionless parameter η_{ER} is introduced to adjust the ratio between calcium influx via the plasma membrane and exchange with the ER according to known physiological data.

Expressions for the calcium fluxes J_{cER} , J_{pER} , and J_{ER} are given by

$$\begin{aligned} J_{\text{cER}} &= -c_1 v_1 m_\infty^3 n_\infty^3 q^3 ([\text{Ca}] - [\text{Ca}]_{\text{ER}}), \\ J_{\text{pER}} &= \frac{v_3 [\text{Ca}]^2}{k_3^2 + [\text{Ca}]^2}, \\ J_{\text{ER}} &= -c_1 v_2 ([\text{Ca}] - [\text{Ca}]_{\text{ER}}), \end{aligned}$$

with $[\text{Ca}]_{\text{ER}}$ being a representative calcium concentration in the ER. q is the activation variable of IP_3 :

$$\begin{aligned} \frac{dq}{dt} &= \alpha_q (1 - q) - \beta_q q, \\ \alpha_q &= a_2 d_2 \frac{[\text{IP}_3] + d_1}{[\text{IP}_3] + d_3}, \\ \beta_q &= a_2 [\text{Ca}]. \end{aligned} \quad (5)$$

m_∞ describes channel activation by IP_3 , and n_∞ is responsible for channel permeability to calcium:

$$\begin{aligned} m_\infty &= \frac{[\text{IP}_3]}{[\text{IP}_3] + d_1}, \\ n_\infty &= \frac{[\text{Ca}]}{[\text{Ca}] + d_5}. \end{aligned}$$

Expressions for the transmembrane calcium fluxes J_{cM} and J_{pM} are given by

$$J_{\text{cM}} = -\frac{1}{2F} (g_{\text{Ca}} m_{\text{Ca}}^4 h_{\text{Ca}} (V_G - V_{\text{Ca}})), \quad (6)$$

$$J_{\text{pM}} = \frac{1}{2F} \frac{g_{\text{pCa}} [\text{Ca}]^2}{k_3^2 + [\text{Ca}]^2}, \quad (7)$$

with the activation functions:

$$\frac{dm_{\text{Ca}}}{dt} = \alpha_{m_{\text{Ca}}} (1 - m_{\text{Ca}}) - \beta_{m_{\text{Ca}}} m_{\text{Ca}}, \quad (8)$$

$$\frac{dh_{\text{Ca}}}{dt} = \alpha_{h_{\text{Ca}}} (1 - h_{\text{Ca}}) - \beta_{h_{\text{Ca}}} h_{\text{Ca}},$$

$$\alpha_{m_{\text{Ca}}} = \frac{0.06(V_G + 19)}{1 - e^{-(V_G + 19)/15}},$$

$$\beta_{m_{\text{Ca}}} = 2.7 e^{-(V_G + 44)/18},$$

$$\alpha_{h_{\text{Ca}}} = 0.045 e^{-(V_G + 49)/18},$$

$$\beta_{h_{\text{Ca}}} = \frac{0.72}{1 + e^{-(V_G + 14)/14}}. \quad (9)$$

Note that the above fluxes are obtained from the corresponding electric currents, divided by the Faraday number and by the valence of the calcium ion.

The calcium concentration in the endoplasmatic reticulum can be written as

$$c_1 W_G \frac{d[\text{Ca}]_{\text{ER}}}{dt} = \eta_{\text{ER}}(-J_{\text{cER}} + J_{\text{pER}} - J_{\text{IER}}), \quad (10)$$

where c_1 characterizes the ratio between the volumes of the cytoplasm and of the endoplasmatic reticulum.

Membrane potential Because the resting potential of the GGC is determined mainly by K^+ ions, our description includes:

- A potassium inward current J_{pump} , which is mainly provided by the active transport into the glial cell by means of the Na^+/K^+ -ATPase and under high extracellular K^+ concentrations by means of the K^+/Cl^- -cotransporter [25]:

$$J_{\text{pump}} = k_{\text{atp}} \frac{[K]_W - [K]_0}{[K]_W + K_{\text{atp}}} + k_{\text{ex}} \frac{[K]_W}{1 + \exp(30.0 - 2[K]_W)}, \quad (11)$$

where the first term represents the relatively weak and constant pumping of K^+ via the Na^+/K^+ -ATPase and the second term is an intensive current via the K^+/Cl^- -cotransporters that are activated by increasing extracellular potassium concentrations (more than 10 mM) [14].

- A potassium outward current provided by K^+ transport through the voltage-dependent K^+ -channels:

$$I_{\text{KGL}} = g_{\text{KGL}} h_{G_\infty} \left(V_G - \frac{RT}{F} \ln \left(\frac{[K]_W}{[K]_G} \right) \right),$$

$$h_{G_\infty} = 0.01 + \frac{1}{1 + e^{(-V_G - 40.0)}}.$$

The expression for activation variable h_{G_∞} was adjusted to fit the known relation between the resting value for the membrane potential of the glial cell V_G and the extracellular potassium concentration $[K]_W$. Namely, it was assumed that, for $[K]_W = 4$ mM, V_G is -72 mV and for $[K]_W = 10$ mM V_G is -50 mV [14].

- Calcium currents I_{pM} , I_{cM} that correspond to the above calcium fluxes J_{pM} , J_{cM} , respectively:

$$I_{\text{pM}} = 2FJ_{\text{pM}}, I_{\text{cM}} = 2FJ_{\text{cM}}. \quad (12)$$

- A leak current $I_{\text{LG}} = g_{\text{LG}}(V_G - V_{\text{LG}})$.

With these different contributions, the equation for GGC transmembrane potential reads:

$$C_{\text{mG}} \frac{dV_G}{dt} = -I_{\text{KGL}} + FJ_{\text{pump}} - I_{\text{pM}} - I_{\text{cM}} - I_{\text{LG}}. \quad (13)$$

Production of neurotransmitter by glial cell Glutamate is released from the glial cell by exocytosis when the cytosolic calcium concentration exceeds a threshold value. To establish a mathematical description of this process, we use the experimental curve by Nadkarni and Jung [7] that describes the whole-cell neuronal current I_{astro} , induced by the glutamate release from astrocyte:

$I_{\text{astro}} = 2.1\Theta\{\ln([\text{Ca}] - 196.69)\}\ln([\text{Ca}] - 196.69)$, where $\Theta\{\}$ is the Heaviside step function, and the calcium concentration is expressed in nanomolars. It is assumed that glutamate release starts when the intragial calcium concentration exceeds the value of ≈ 200 nM.

In our work, we assume that the shape of the described curve is determined by nonlinear features of glial glutamate release rather than by the neuronal sensitivity to glutamate. Thus, to describe the relation between cytosolic calcium concentration in the GGC and glial glutamate release to the intercellular volume W [see (2)], we approximate the data from [7] with a slightly more complicated but smooth function M_G :

$$M_G = r_{\text{GluG}}\Psi([\text{Ca}])\frac{[\text{Glu}]_G}{([\text{Glu}]_G + K_{\text{GluG}})}, \quad (14)$$

$$\Psi([\text{Ca}]) = \frac{1}{(1 + \exp\{-2\delta_{\text{Ca}}/(1 + 50/(1 + \exp(-2\delta_{\text{Ca}})))\})} \quad (15)$$

$$\delta_{\text{Ca}} = 1000[\text{Ca}] - 196.69. \quad (16)$$

Here, δ_{Ca} measures how much the intragial calcium concentration exceeds the threshold level in nanomolars, K_{GluG} represents the Michaelis constant for this process, and r_{GluG} characterizes the maximal rate of glutamate release.

Note that the release of glutamate from the GGC occurs in a discrete manner and includes both (1) the release of mediator depending on cytosolic calcium concentration and (2) the amount of glutamate in vesicles ready to be released. The expression for M_G approximates the first part of the curve. To also describe the second part of the curve, the following equation was used:

$$W_G \frac{d[\text{Glu}]_G}{dt} = -M_G + r_{\text{GluG}_0} \frac{[\text{Glu}]_{G_0} - [\text{Glu}]_G}{([\text{Glu}]_{G_0} - [\text{Glu}]_G) + [\text{Glu}]_{G_0}/2}, \quad (17)$$

where r_{GluG_0} characterizes the rate of the formation of vesicles with glutamate in W_G and Glu_{G_0} is the equilibrium glutamate concentration.

3 Simulation Results

By its formulation, our model will be able to reproduce the reference values of some of the variables, known from experimental studies. For example, the rise of the extracellular potassium from 4 mM (equilibrium value) to 10 mM that provides the shift of the glial cell membrane potential to ≈ -50 mV, which, in turn, opens the voltage-gated calcium channels in the glial membrane. However, other quantities in the model can only be estimated and set to some typical values. One must keep in mind that only the structurally stable regimes

might be relevant to a real living system. This means that life-relevant operating regimes of the system have to be resistant to a reasonable variation of many control parameters, as well as to fluctuations of the environment.

Keeping all this in mind, we illustrate below the typical responses of our model and discuss its features as a result of the interplay between the nonlinearities and time scales of the involved processes.

3.1 Activation of the Glial Cell

In the numerical simulations to be presented in this section, the glutamate release from the GGC was blocked by setting $r_{\text{GluG}} = 0$. This implies that the calcium response of the GGC can be evoked, but it has no effect on the postsynaptic R-neuron.

First, we test how the synaptic transmission between the P- and R-neurons gives rise to changes of the concentrations in the intercellular volume W , which, in its turn, affects the operation regime of the glial cell. The time courses of the relevant model variables are shown in Fig. 2. The two top panels in this figure show the spike train of the mechanosensory P-neuron, triggered by an external stimulus (filled bar in the figure), and the evoked response of the R-neuron. There is a considerable time lag between the spike trains in P- and R-neurons due to integrative features of this particular synapse [26–28]. The two following panels in Fig. 2 illustrate the corresponding changes that take place in the extracellular space. Namely, there is a rise of the glutamate concentration $[\text{Glu}]$ that results from the glutamate release from the activated presynaptic terminal.

Note that $[\text{Glu}]$ starts to decrease while the P-neuron is still active. This fact indicates that the firing rate of the P-neuron considerably influences the balance between the influx of glutamate from the synapse and its degradation due the diffusion to the surrounding bath solution and, probably, other mechanisms. A similar effect is observed for the extracellular potassium concentration $[\text{K}]_W$. It rises with some time lag relative to the glutamate curve, which is roughly equal to the time lag between the spike train of the two neurons, and starts to gradually decrease when the firing rate of R-neuron decreases a little.

Thus, we observe that the synaptic transmission between the P and R neurons is accompanied by rapid changes of the glutamate and potassium concentration in the extracellular space. The lowest three panels in Fig. 2 characterize the corresponding glial response.

The elevation of the $[\text{K}]_W$ shifts the corresponding Nernst potential for the glial cell and, consequently, the resting glial membrane potential V_G . According to experimental evidence, V_G is mainly defined by the potassium balance. Thus, the rate of changes in V_G being high, its instant value follows the changes in $[\text{K}]_W$. The time course of V_G shows the rapid depolarization of the glial cell. The reference values for $[\text{K}]_W$ and V_G (10 mM and -50 mV, respectively) are indicated in Fig. 2 with horizontal dashed lines. According to the experimental data, when these variables exceed the given reference values, a rapid and intensive calcium influx to the glial cell occurs. The time course of the $[\text{Ca}^{2+}]$ displays this effect. The maximal value for calcium elevation is controlled by many parameters. It is increased by longer stimuli or faster rise (slower degradation) of the extracellular potassium concentration or by higher membrane conductance for calcium, etc. For the given set of control parameters and stimulus duration, the maximal calcium concentration shown in Fig. 2 is lower than the threshold value ($\approx 0.2 \mu\text{M}$) for the glial glutamate release. It is important to note that the IP_3 production triggered by the extracellular glutamate elevation

Fig. 2 Time course for the model variables (from top to the bottom): transmembrane voltage V_P and V_R for P and R neurons, respectively; extracellular glutamate concentration $[Glu]$; extracellular potassium concentration $[K]_w$; glial cell membrane potential V_G ; glial cytosolic calcium $[Ca]$; and the intragial IP_3 concentration. The filled horizontal bar above the top panel indicates the duration of the external stimulus applied to the P-neuron

(bottom panel) has no pronounced effect on the calcium concentration; this despite the fact that the corresponding rate factor r_g was chosen to be a little higher than the maximal realistic rates estimated in [23]. We can therefore conclude that the glial activation pathway for a GGC considerably differs from the one reported previously for astrocytes [7, 23, 24].

3.2 Tripartite Synapse in Action

To investigate numerically the features of glial-mediated synaptic transmission in what is called a tripartite synapse, we unblock both the glutamate release from the glial cell, by setting a nonzero r_{GluG} , and the sensitivity of the R-neuron to glutamate. Now, the extracellular concentration of glutamate is mediated by both the presynaptic neuron and the glial cell.

With these changes, activation of the calcium subsystem in the glial cell may now influence both the activity of the postsynaptic neuron and the glial cell's own glutamate-triggered IP_3 production.

The representative time courses for the selected model variables are shown in two groups of panels in Fig. 3. The top four panels show the result of relatively short (during 150 ms) stimulation of the P-neuron. Stimulus was applied at $t = 200$ ms and removed at $t = 350$ ms (indicated by the filled bar in the figure). One observes qualitatively the

Fig. 3 With unblocked glutamate production by the glial cell, a short stimulation of the P-neuron does not result in a glial calcium response (*top panels*), while the longer stimulation triggers glial glutamate release and evokes self-sustained neuronal firing (*bottom panels*). Dotted curves in the bottom panels show the glial and neuronal response at 8% lower sensitivity of the R-neuron to the extracellular glutamate concentration

same results as before: there is a pronounced rise of extracellular glutamate and potassium concentration, as well as of intragial calcium. All these processes decay quickly when the R-neuron stops firing. However, the longer stimulus (during 200 ms) reveals a different behavior, as illustrated in the four bottom panels of Fig. 3. Namely, as soon as a calcium concentration $[Ca]$ exceeds the threshold value of $\approx 0.2 \mu M$, the glutamate release from the glial cell is triggered. The threshold calcium level and the corresponding moment of time are indicated by dashed lines in the figure. This event is responsible for the rapid rise of extracellular glutamate that provides an additional depolarizing current for the R-neuron. Thus, the R-neuron maintains continuous spiking behavior even after silencing of the P-neuron. The spiking activity of the R-neuron, in turn, maintains the extracellular potassium concentration above the 10-mM level that keeps V_G above -50 mV and thus provides the further release of glutamate. In the bottom panels of Fig. 3, the transition to such a self-sustained regime is clearly indicated by the elevation of glutamate, potassium, and calcium concentration followed by its stabilization at a certain level. After some time, the slowly increasing interglial IP_3 concentration can also contribute to the processes.

The observed transition to self-sustained firing is controlled by many parameters. For example, in the considered regime, the self-sustained firing can be terminated by reducing the R-neuron's sensitivity to glutamate. This is illustrated by the dotted curves in the bottom panels of Fig. 3. The same effect can be achieved by increasing the rate of glutamate diffusion (equal to the diffusion rate of K^+ ions). The behavior depends on the relation between the contribution from internal calcium stores and from the transmembrane calcium influx. However, because the effect under consideration is actually provided by the positive feedback loop, the action of different parameters is similar: they can support or suppress the activation of what we call the "glutamate loop." On this basis, the observed effect is structurally stable and originates from the nature of interaction pathways in the considered neural–glial ensemble.

Fig. 4 At longer time scales, the self-sustained firing of the R-neuron is terminated as the glial glutamate store is emptied

It is well known that positive feedback generally makes a system unstable. The stable self-controlled operation regime of a system is typically provided by negative feedback loops. In our case, a positive feedback switches the neural–glial ensemble to a state of continuous firing, which is undesirable in the present system. Thus, some additional mechanisms that can suppress the self-sustained firing of the R-neuron should be taken into account. In the present work, we assume that a finite amount of glutamate is stored in the glial cytosol in the form of exocytotic vesicles. With this assumption, the self-sustained elevation of the extracellular glutamate concentration continues as long as the intragial glutamate store is not emptied. In the model, this is described by (17). The representative simulation results are shown in Fig. 4. One can see that the course of self-sustained continuous firing is terminated at approximately 7,500 ms, when the $[Glu]_G$ becomes zero. Evidently, the possible duration of a subsequent continuous firing event depends on the time interval of refilling of interglial glutamate stores.

4 Conclusions

We have presented a detailed model of the role of the GGC in synaptic transmission within leech ganglia. By comparison with previously reported models of the neuron–glia system, we considered different activation pathways of the glial cell. In particular, our model includes an increase in extracellular potassium concentration, which provides the depolarization of the glial cell and thus triggers the calcium influx through the voltage-gated calcium channels.

We indicated the existence of a positive feedback loop that is responsible for a continuous self-sustained activity of the postsynaptic neuron and a prolonged activation of the glial cell. Finally, we suggest that the mechanism terminating the self-sustained tonic firing is related to the emptying of the intragial glutamate store.

In future work on the model, we plan to include (1) other pathways of neuron-to-glia and neuron-to-neuron signaling via serotonin (5-HT) released from activated the R-neuron and (2) additional Ca^{2+} fluxes into the glial cell through ionotropic glutamate receptors and via Ca^{2+} -channels in ER induced by interaction of 5-HT with its metabotropic receptors [14]. The first addition will allow us to study how a complicated net of positive and negative feedback loops affect all the components of a tripartite synapse and to predict pathological states more precisely. The second addition will extend our understanding of processes in glial cells and broaden the applicability of the model to mammalian neuroglial ensembles.

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5 Appendix

Parameters and constants for GGC model

Symbol	Definition	Value and units
W_G	Glial cytosolic volume per unit surface area	1.0 nL/cm^2
r_g	Rate of glutamate-dependent IP_3 production	$0.0001 \mu\text{M/ms}$
$[\text{IP}_3]_{\text{eq}}$	Equilibrium IP_3 concentration	$0.160 \mu\text{M/L}$
τ_{IP_3}	Time scale for IP_3 production	71486.0
η_{ER}	ER/membrane contribution factor	Control parameter $\in [0.0; 1.0]$
γ_G	Diffusion rate per unit length for cytosolic calcium	$0.01 \text{ nL}/(\text{cm}^2 \cdot \text{ms})$
$[\text{Ca}]_0$	Equilibrium inter-glial calcium concentration	$0.06 \mu\text{M}$
g_{Ca}	Calcium conductance of voltage-gated channels	400 mS/cm^2
g_{pCa}	Equivalent conductance of Ca^{2+} ATPase	1 mS/cm^2
g_{KG}	Potassium conductance	600 mS/cm^2
$[\text{K}]_G$	Inter-glial potassium concentration	72 mM
k_{atp}	Rate of ATP-dependent potassium pumping into the glial cell	$0.01 \mu\text{A}/(\text{cm}^2 \cdot \text{ms})$
K_{atp}	Michaelis constant for ATP-dependent potassium pumping into the glial cell	0.9 mM
k_{ex}	Rate of chloride-dependent potassium uptake into the glial cell	1.0 mM/cm^2
C_{mG}	Glial cell membrane capacitance per unit surface area	$1.0 \mu\text{F/cm}^2$
c_1	Ratio of effective volumes between the cytoplasm and ER	0.185
v_1	Rate of calcium flux from ER through IP_3 -sensitive channels	$6.0/\text{ms}$
v_2	Rate of leaky calcium flux from ER	$0.11/\text{ms}$
v_3	Maximal rate of calcium uptake into ER	$0.9 \mu\text{M}/(\text{L/ms})$
k_3	Michaelis constant for calcium uptake into ER	$0.1 \mu\text{M}$
a_2	Rate factor for q variable	0.2
d_1	Parameter of q activation function	$0.13 \mu\text{M}$
d_2	Parameter of q activation function	$1.049 \mu\text{M}$
d_3	Parameter of q activation function and instant activation of m_∞	$0.9434 \mu\text{M}$
d_5	Parameter of instant activation of n_∞	$0.08234 \mu\text{M}$
r_{GluG}	Rate of calcium-dependent glial glutamate release	Control parameter, $[0.0; 0.1] \text{ nL} \cdot \text{mM}/(\text{cm}^2 \cdot \text{ms})$
k_{GluG}	Michaelis constant of calcium-dependent glial glutamate release	$0.01 \text{ nL} \cdot \text{mM}/\text{cm}^2 \cdot \text{ms})$
r_{GluG0}	Rate for intra-glial glutamate refilling and production	Control parameter, $[0.01; 0.02] \text{ nL} \cdot \text{mM}/(\text{cm}^2 \cdot \text{ms})$
$[\text{GluG}_0]$	Intra-glial equilibrium glutamate concentration	Control parameter, mM

Other parameters and constants (Cont.)

Symbol	Definition	Value and units
W	Extracellular volume per cellular surface area	1.0 nl/cm ²
$[K]_0$	Extracellular potassium bath concentration	4.0 mM
γ_W	Diffusion coefficient for potassium per unit length	0.01 nl/(cm ² · ms)
r_s	Glutamate release intensity	0.3 mM · nL/(cm ² · ms)
γ_G	Degradation factor for glutamate based on diffusion coefficient per unit length	0.01 nl/(cm ² · ms)
$[Glu]_0$	Glutamate bath concentration	0.0 mM
R	Universal gas constant	8.315 J/(mol · K)
T	Normal temperature	293.15 K
F	Faraday's constant	96.49×10^3 C/mol

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